Election and Reply to Restriction

to Office Action dated April 8, 2008

Atty. Docket No: 56029-51044

Listing of claims:

1. (Previously presented) A live attenuated derivative of a pathogenic Salmonella species consisting essentially of

(a) a means for regulatable expression of a gene that encodes a regulatory protein, wherein non-expression of said regulatory protein in vivo causes synthesis of a first antigen that is conserved among *Salmonella* species and *E. coli* strains; and

(b) a means for regulatable synthesis of a first carbohydrate antigen, wherein said first carbohydrate antigen ceases to be synthesized in vivo, exposing a second carbohydrate antigen that is conserved among *Salmonella* species and *E. coli* strains;

wherein said attenuated derivative has enhanced ability to induce cross-protective immunity against *Salmonella* species and *E. coli* strains.

2. (Previously presented) The live attenuated derivative of claim 1, further comprising a means for non-expression of a serotype-specific antigen.

3. (Previously presented) The live attenuated derivative of claim 2, wherein said means for non-expression of a serotype-specific antigen comprises a mutation in a gene selected from the group consisting of fliC and fljB.

4. (Previously presented) The live attenuated derivative of claim 3, wherein said mutation is a deletion mutation.

5. (Previously presented) The live attenuated derivative of claim 1, wherein said means of regulatable expression comprises substituting the promoter of said gene that encodes a regulatory protein with a regulatable promoter.

6. (Previously presented) The live attenuated derivative of claim 5 wherein said regulatable promoter is the *araCP*_{BAD} repressor-activator-promoter system.

2

Election and Reply to Restriction

to Office Action dated April 8, 2008

Atty. Docket No: 56029-51044

7. (Previously presented) The live attenuated derivative of claim 6 wherein said gene that

encodes a regulatory protein is selected from the group consisting of fur, rpoS, phoPQ, dam,

ompR, cya and crp.

8. (Previously presented) The live attenuated derivative of claim 1 wherein said carbohydrate

antigen is an LPS O-antigen.

9. (Previously presented) The live attenuated derivative of claim 8 wherein said means for

regulatable synthesis comprises a mutation in a gene that encodes a product necessary for

synthesis of LPS O-antigen.

10. (Previously presented) The live attenuated derivative of claim 9, wherein said means for

regulatable synthesis comprises a mutation in the pmi gene.

11. (Previously presented) A method for inducing an immune response sufficient for protection

against infection by Salmonella species and E. coli strains, said method comprising administering

to an individual the live attenuated derivative of claim 1.

12. (Previously presented) A live attenuated derivative of a pathogenic Salmonella species,

consisting essentially of

(a) a means for regulatable expression of a fur gene; and

(b) a mutation that renders a pmi gene inoperable,

wherein said attenuated derivative has enhanced ability to induce cross-protective

immunity against Salmonella species and E. coli.

13. (Previously presented) The live attenuated derivative of claim 12 wherein said means of (a)

comprises substituting the fur promoter with a regulatable promoter.

Election and Reply to Restriction

to Office Action dated April 8, 2008 Atty. Docket No: 56029-51044

14. (Previously presented) The live attenuated derivative of claim 12, wherein said means of (a)

comprises replacing the fur promoter with the araCP_{BAD} activator-repressor-promoter system.

15. (Previously presented) The live attenuated derivative of claim 12 wherein said means of (a)

comprises the $\Delta Pfur::araCP_{BAD}fur$ genetic construction.

16. (Previously presented) The live attenuated derivative of claim 12 wherein said mutation of

(b) is a deletion mutation.

17. (Previously presented) A method of inducing a cross-protective immune response against

Salmonella species, said method comprising administering to an individual the live attenuated

derivative of claim 2.

18. (Canceled)

19. (Previously presented) A vaccine comprising a live attenuated strain of Salmonella, wherein

said live attenuated strain consists essentially of

(a) a mutation in a pmi gene that renders said pmi gene non functional; and;

(b) a genetic construction that allows for regulatable expression of a fur gene,

wherein said vaccine has enhanced ability to stimulate cross protective immunity against

Salmonella species and E. coli strains.

20. (Canceled)

21. (Previously presented) A vaccine comprising a live attenuated strain of Salmonella, wherein

said live attenuated strain consists essentially of

(a) a mutation that renders a pmi gene non functional; and

Election and Reply to Restriction

to Office Action dated April 8, 2008

Atty. Docket No: 56029-51044

(b) a regulatable promotor operably linked to a *fur* gene wherein said *fur* gene is expressed when said attenuated strain is in the intestinal tract of an individual and said *fur* gene is

not expressed when said attenuated strain is within internal tissues of an individual.

22. (Previously presented) The vaccine of claim 21 wherein said regulatable promoter

comprises the $araCP_{BAD}$ activator-repressor-promoter system.

23. (Previously presented) A live attenuated derivative of a Salmonella species consisting

essentially of

(a) a means for regulatable synthesis of LPS O-antigen side chains, wherein said O-

antigen side chains are synthesized when said attenuated derivative is in the intestinal tract of an

individual and are not synthesized when said attenuated derivative is within internal tissues of an

individual; and

(b) a means for regulatable expression of a fur gene, wherein said fur gene is expressed

when said attenuated derivative is in the intestinal tract of an individual and wherein said fur

gene is not expressed when said attenuated derivative within internal tissues of an individual

wherein said attenuated derivative has increased ability to induce cross-protective

immunity against infection by Salmonella species and E. coli strains.

24. (Previously presented) The live attenuated derivative of claim 23 wherein said means for

regulatable synthesis comprises a mutation in a gene that encodes a product necessary for

synthesis of LPS O-antigens.

25. (Previously presented) The live attenuated derivative of claim 24 wherein said gene that

encodes a product necessary for synthesis of LPS O-antigens is a pmi gene.

26. (Previously presented) A live attenuated derivative of a Salmonella typhimurium comprising

(a) a ΔPfur::TTaraCP_{BAD}fur deletion-insertion mutation; and

(b) a Δpmi mutation

4732484.1 5

Election and Reply to Restriction

to Office Action dated April 8, 2008

Atty. Docket No: 56029-51044

27. (Previously presented) A recombinant bacterial strain consisting essentially of a means of

regulatable expression of a virulence gene, wherein said regulatable expression of a virulence

gene renders said bacterial strain attenuated while maintaining immunogenicity.

28. (Previously presented) The recombinant Salmonella of claim 27, wherein said virulence

gene is selected from the group consisting of aroA, aroC, aroD, cya, crp, cdt, ompR, htrA, hemA,

purA, purB, rfa, rfb, asd ompC and ompF.

29. (Previously presented) The recombinant bacterial strain of claim 27, wherein said means of

regulatable expression comprises substituting the promoter for said virulence gene with the

 $araCP_{BAD}$ repressor-activator-promoter system.

30. (Previously presented) The recombinant bacterial strain of claim 29, wherein said virulence

gene is a fur gene.

31. (Previously presented) The recombinant bacterial strain of claim 30, further comprising a

 Δpmi mutation.

32. (Previously presented) A live attenuated derivative of a pathogenic Enterobacteriaceae

species consisting essentially of a ΔP fur:: $araCP_{BAD}$ fur genetic construction.

33. (Previously presented) A live attenuated derivative of a pathogenic Salmonella species

consisting essentially of

(a) a means for regulatable expression of a gene that encodes a regulatory protein,

wherein non-expression of said regulatory protein in vivo causes synthesis of a first antigen that

6

is conserved among Salmonella species and E. coli strains; and

Election and Reply to Restriction

to Office Action dated April 8, 2008

Atty. Docket No: 56029-51044

(b) a means for regulatable synthesis of a first carbohydrate antigen, wherein said first carbohydrate antigen ceases to be synthesized in vivo, exposing a second carbohydrate antigen that is conserved among *Salmonella* species and *E. coli* strains; and

(c) a mutation of *fliC* or *fljB*, wherein said mutation results in deletion of the variable domain while retaining the N-terminal and C-terminal constant domains of flagellar proteins;

wherein said attenuated derivative has enhanced ability to induce cross-protective immunity against *Salmonella* species and *E. coli* strains.

- 34. (Previously presented) The live attenuated derivative of claim 1, further comprising a means for biological containment.
- 35. (Previously presented) The live attenuated derivative of claim 34, wherein said means comprises a mutation that abolishes motility, prevents synthesis of the exopolysaccharide colanic acid, prevents synthesis of components of the bacterial extracellular matrix, reduces ability to withstand the stresses of stationary phase and starvation, reduces ability to use nucleic acids as a nutrient, or uncouples regulation of cellular activities from a dependence on protein synthesis.
- 36. (Previously presented) The live attenuated derivative of claim 35, wherein said mutation is selected from the group consisting of $\Delta(gmd\text{-}fcl)$ -26, $\Delta agfBAC811$, $\Delta bcsABZC2118$, $\Delta bcsABZC2119$, $\Delta adrA1418$, $\Delta mlrA34$, $\Delta yhiR36$::TT, $\Delta endA2311$, $\Delta relA1123$.
- 37. (Previously presented) The live attenuated derivative of claim 35, wherein said mutation consists of a mutation in a gene selected from the group consisting of *gmd*, *fcl*, *agf*, *bcs*, *adr*, *mlr*, *yhi*, *end* and *rel*.
- 38. (Previously presented) The live attenuated derivative of claim 1, further comprising a mutation in a gene selected from the group consisting of *sip* and *sop*.

Appl. No. 10/511,616 Election and Reply to Restriction

to Office Action dated April 8, 2008

Atty. Docket No: 56029-51044

39. (Previously presented) The live attenuated derivative of claim 38, wherein said mutation is $\Delta sop B1925$.

40. (Previously presented) The live attenuated derivative of claim 1, wherein said live attenuated derivative comprises the $\Delta ilvG3$::TTaraCP $_{BAD}lacI$ genetic construction.